Lentivirus Packaging Mix

Cat#LV003, LV053

The following protocol allows the production of recombinant lentiviral vector up to 10^7 IU/mL. We recommend including a negative control (without DNA nor transfection reagents) in your experiment to help evaluate your results. You will need 1.2 x 10^7 293T cells for each sample.

Before starting the lentiviral packaging protocol, please ensure you have ample expression DNA. A DNA amplification step may be required using standard bacterial transformation protocols. It is important to note that *E. coli* DH5-alpha strains have been tested to produce optimal plasmids for lentiviral packaging.

DAY 1:

1. Seed 293T cells into 10cm dishes (add ~1.2 X 10^7 cells per 10cm dish).

DAY 2:

2. Check to make sure the cells are 80-90% confluent.
3. For each 10cm dish prepare transfection complex as follows:
    a. Solution A: Dilute 20µg DNA plasmids (10µg expression vector and 10µg of Packaging Mix) in 1mL serum-free medium
    b. Solution B: Dilute 80µL of LentiFectin™ reagent in 1mL serum-free medium
    c. Incubate both solutions at room temperature for 5 minutes.
    d. Mix Solution A and Solution B together and incubate at room temperature for 20 minutes. This is the transfection complex.
4. Add 4.5mL serum-free medium to the transfection complex.
5. Remove medium from the cells.
6. Add the complete transfection complex from step 4 to the cells and incubate at 37°C for 5-8 hours.
7. Add 0.65mL FBS to each dish and incubate at 37°C overnight.

DAY 3:

8. Remove the old medium from the cells.
9. Add 10mL complete culture medium to the cells.

10. Incubate at 37°C for 24 hours.

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DAY 4 (Harvest):

11. Collect supernatant medium from the culture dish.
12. Centrifuge supernatant at 3000 rpm for 15 minutes at 4°C to pellet debris.
13. Transfer the cleared supernatant to a new tube. Filter the cleared supernatant with a low-protein binding 0.45 μg sterile filter.
14. The virus is ready for infection, purification, or it can be stored as a viral stock at -80°C for your future applications. Aliquot volumes are preferred for storage to reduce the viral titer loss from freeze-thaw cycles.
15. A second harvest can be done by adding 10mL of complete medium to the cells after the first harvest.
16. Second harvest can be done on Day 5, following steps 11-13.

Note: Expression of the VSVG glycoprotein causes 293T cells to fuse, resulting in the appearance of large, multinucleated cells known as syncytia. This morphological change is normal and does not affect the production of the lentivirus.